abcam

Product datasheet

Anti-Smad3 antibody [EP568Y] ab40854

KO VALIDATED Recombinant RabMAb

10 Abreviews 271 References 21 Images

Overview

Product name	Anti-Smad3 antibody [EP568Y]
Description	Rabbit monoclonal [EP568Y] to Smad3
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, IHC-P, Sandwich ELISA, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Rat, Human **Predicted to work with:** Mouse, Chicken, Pig
Immunogen	Synthetic peptide within Human Smad3 aa 200-300 (internal sequence). The exact sequence is proprietary. Database link: **P84022** (Peptide available as **ab173094**)
Positive control	WB: A549, HeLa, Human Kidney, HT-29, HT-1080 and Jurkat whole cell lysates; Rat liver tissue lysate. IHC-P: Human prostate carcinoma, breast carcinoma, colonic adenocarcinoma, lung adenocarcinoma, gastric adenocarcinoma, glioma and liver tissues. ICC/IF: HepG2 cells. Flow Cyt (intra): HCT116 and HT-29 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP568Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40854 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50 - 1/210. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
WB	★★★★☆ (7)	1/1000 - 1/10000. Detects a band of approximately 55 kDa (predicted molecular weight: 48 kDa).
IHC-P	★★★★★ (2)	1/500 - 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Sandwich ELISA		Use a concentration of 0.5 µg/ml. For sandwich ELISA, use this antibody as Detection at 0.5 µg/ml with Mouse monoclonal [AF9F7] to Smad3 (**ab75512**) as Capture.
ICC/IF	★★★★★ (1)	1/500 - 1/2000.

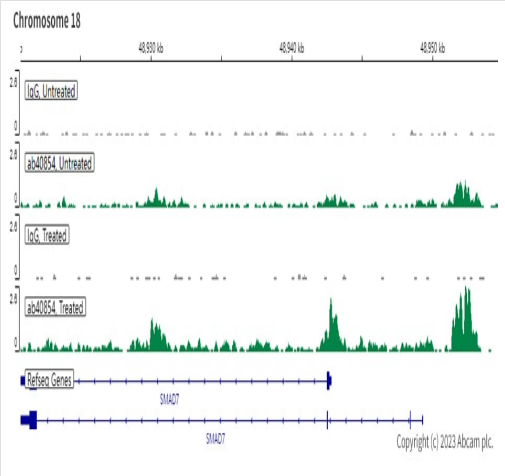
Application notes Is unsuitable for IP.

Target

Function Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

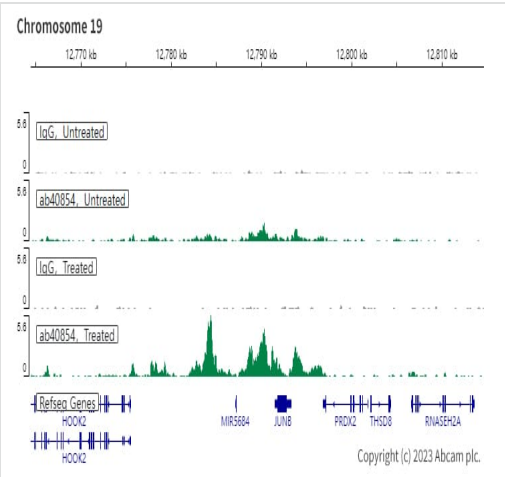
Involvement in disease	Colorectal cancer Loeys-Dietz syndrome 3
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Domain	The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import. The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain.
Post-translational modifications	Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta. Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes. Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

Images



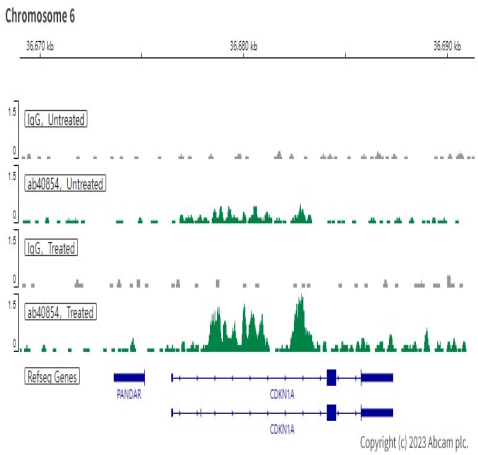
ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab40854 [EP568Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



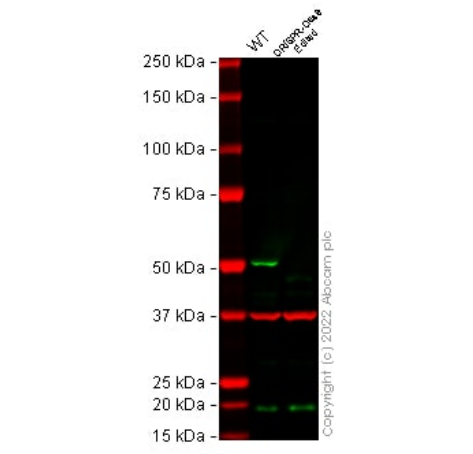
ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab40854 [EP568Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-Smad3 antibody [EP568Y] (ab40854)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/µL, 2.5 x 10^5 A549 (Human lung carcinoma cell line) cells treated with hTGF-β1 (7 ng/mL 1 h) and 5 µg of ab40854 [EP568Y]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

All lanes : Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : SMAD3 CRISPR-Cas9 edited A549 cell lysate

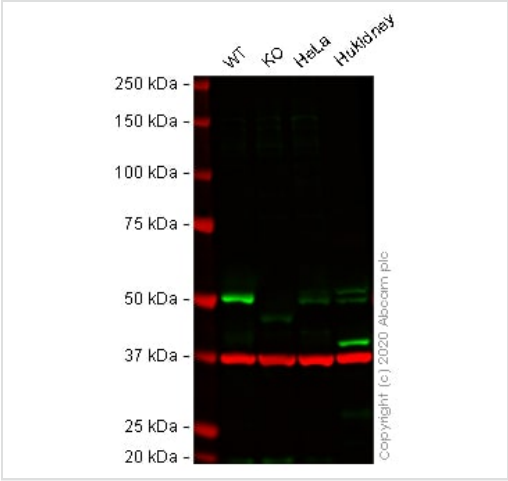
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 50 kDa

False colour image of Western blot: Anti-Smad3 antibody [EP568Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40854 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type A549 cell lysates with no signal observed at this size in SMAD3

CRISPR-Cas9 edited cell line **ab277888** (CRISPR-Cas9 edited cell lysate None). The band observed in the CRISPR-Cas9 edited lysate lane below 50 kDa is likely to represent a truncated form of Smad3. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and SMAD3 CRISPR-Cas9 edited A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

All lanes : Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human Kidney cell lysate

Lysates/proteins at 20 µg per lane.

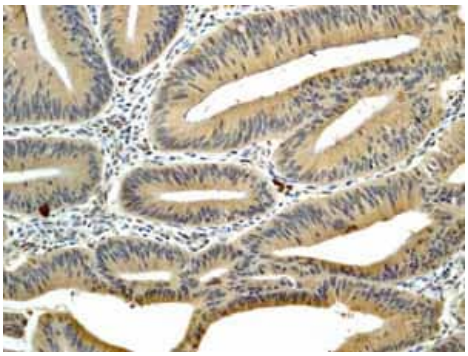
Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab40854 observed at 48 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab40854 was shown to react with Smad3 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab40854 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-

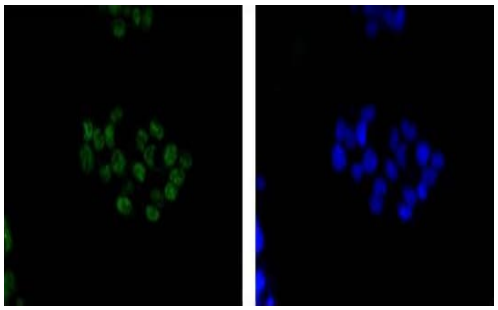
Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

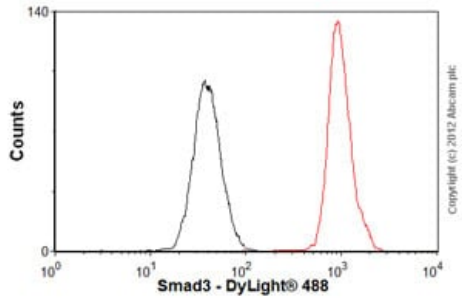
Immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue labelling Smad3 with unpurified ab40854.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



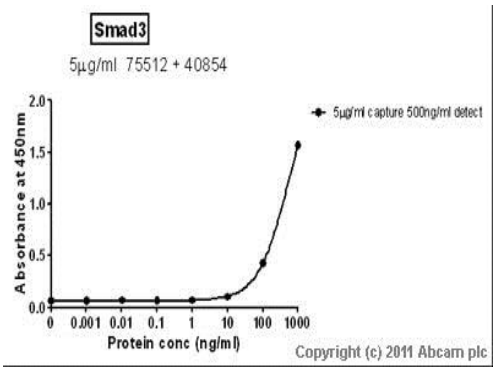
Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunocytochemsitry/Immunofluorescence analysis of HepG2 cells labelling Smad3 (green) with purified ab40854 at 1/2000 . Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody (green, left panel). Counterstained with DAPI (blue, right panel).



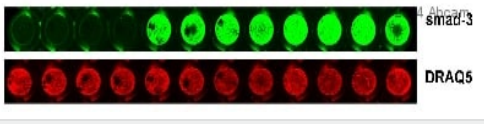
Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] (ab40854)

Overlay histogram showing HCT116 cells stained with unpurifiedab40854 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40854, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Sandwich ELISA - Anti-Smad3 antibody [EP568Y] (ab40854)

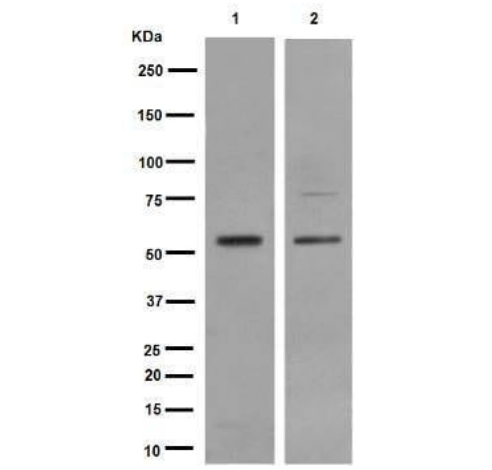
Standard Curve for Smad3 (Analyte: **Smad3 protein (His tag) (ab89353, unpurified)**); dilution range 1pg/ml to 1µg/ml using Capture Antibody **Mouse monoclonal [AF9F7] to Smad3 (ab75512)** at 5µg/ml and Detector Antibody **Rabbit monoclonal [EP568Y] to Smad3 (ab40854)** at 0.5µg/ml.



Immunocytochemistry/ Immunofluorescence - Anti-Smad3 antibody [EP568Y] (ab40854)

This image is courtesy of an Abreview submitted by Francesco Elia Marino

ab40854 staining Smad3 in human granulosa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with ethanol and triton and blocked for 1 hour at 26°C. Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. An undiluted IRDye® 800CW-conjugated goat anti-rabbit IgG (H+L) polyclonal was used as the secondary antibody. Left - negative control (4 replicates).



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

All lanes : Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5300 dilution (purified)

Lane 1 : HT-29 cell lysate
Lane 2 : HT-1080 cell lysate

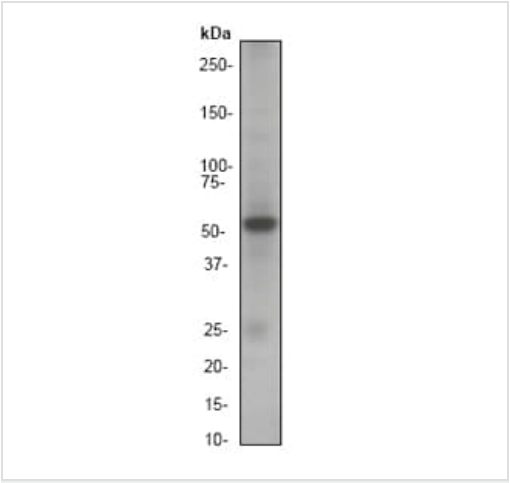
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 48 kDa
Observed band size: 58 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

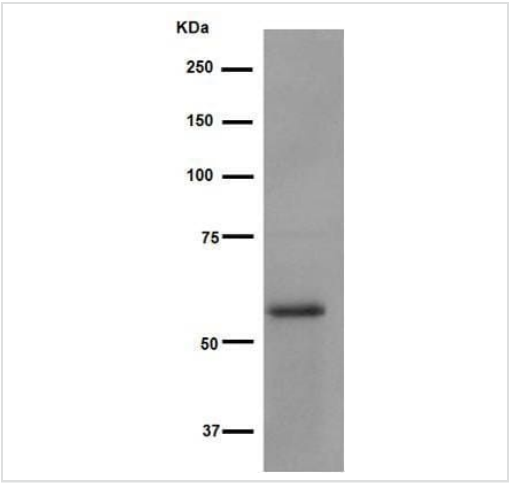
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5000 dilution (unpurified) + Jurkat cell lysate at 10 µg

Predicted band size: 48 kDa
Observed band size: 55 kDa



Western blot - Anti-Smad3 antibody [EP568Y] (ab40854)

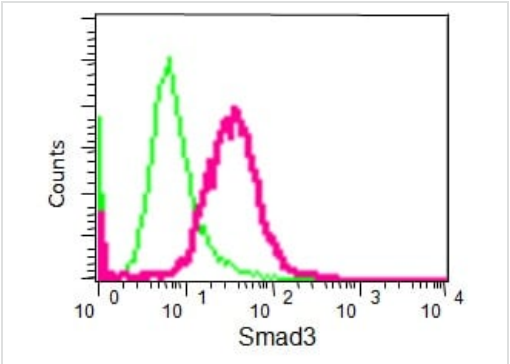
Anti-Smad3 antibody [EP568Y] (ab40854) at 1/5300 dilution (purified) + Rat liver tissue lysate at 10 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 48 kDa
Observed band size: 58 kDa

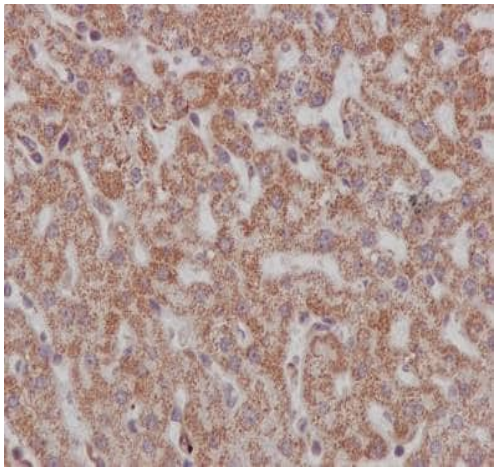
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



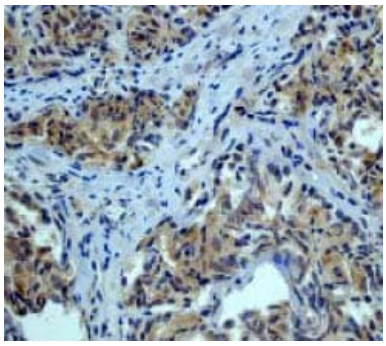
Flow Cytometry (Intracellular) - Anti-Smad3 antibody [EP568Y] (ab40854)

Intracellular Flow Cytometry analysis of HT-29 cells labelling Smad3 with purified ab40854 at 1/210 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

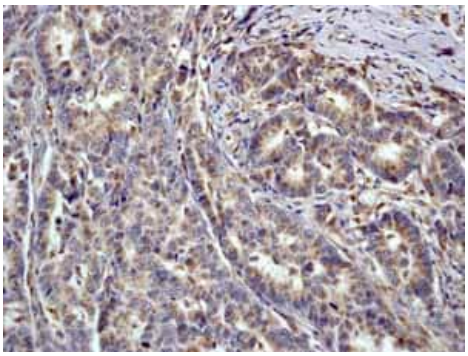
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Smad3 with purified ab40854 at 1/2000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling unpurified ab40854 at 1/100 dilution.

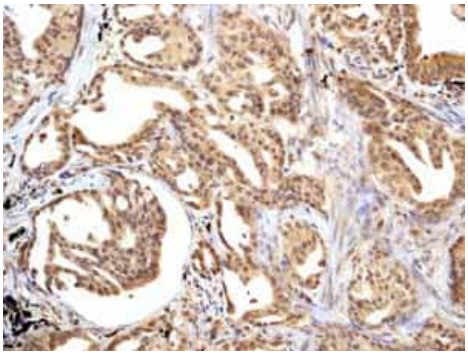
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling unpurified ab40854.

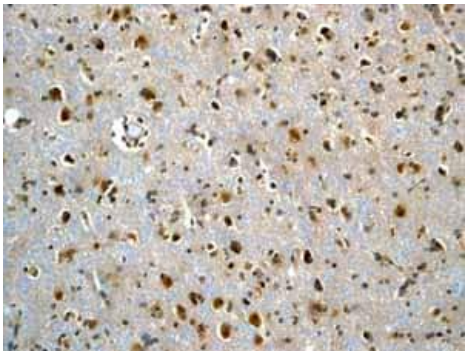
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung adenocarcinoma tissue labelling unpurified ab40854.

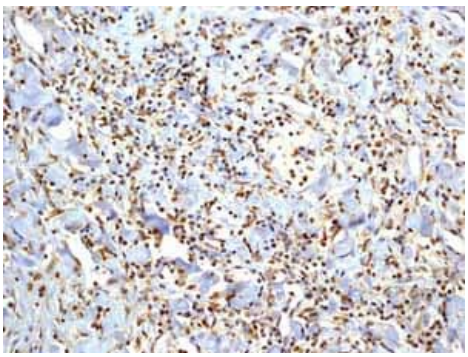
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue labelling unpurified ab40854.

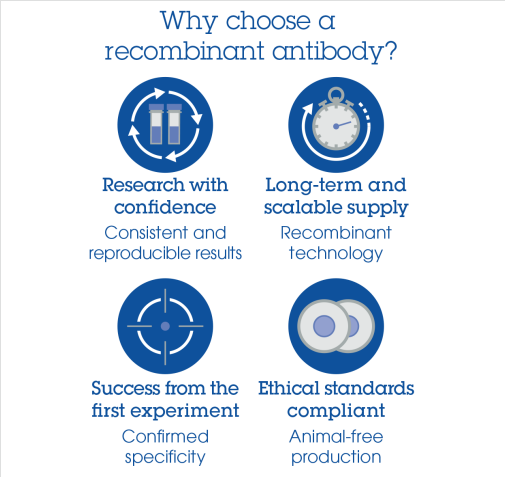
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad3 antibody [EP568Y] (ab40854)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labelling unpurified ab40854.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Smad3 antibody [EP568Y] (ab40854)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors